ELSEVIER

Behavioural Brain Research 64 (1994) 9–14

BEHAVIOURAL BRAIN RESEARCH

Role of thyroid hormones in the maturation of interhemispheric connections in rats[1]

Pere Berbel*, Ana Guadaño-Ferraz, Antonia Angulo, José Ramón Cerezo

Departament d'Histologia, Facultat de Medicina i Institut de Neurociències, Universitat d'Alacant, Apartat de Correus 374, 03080 Alacant, Spain

Received 1 February 1993; revised 14 July 1993; accepted 23 February 1994

Abstract

Hipothyroidism causes mental retardation secondary to changes in the organization of the CNS. These changes affect higher brain functions for which interhemispheric transfer of information is crucial. In present study, the anterior commissure (AC) and corpus callosum (CC) of normal (C) and hypothyroid (H) rats has been examined using quantitative electron microscopy. H rats received an antithyroid treatment with methimazole from embryonic day 14 (E14) and surgical thyroidectomy at postnatal day 6 (P6). In the AC, the number of axons (unmyelinated and myelinated) increased from 0.17×10^6 axons at E18 to 1.08×10^6 axons at P4 and it was almost the same at P180 (1.01×10^6 axons). In H rats the number of axons between P14 and P180 was similar to that of C rats. In contrast, there were only 0.11×10^6 myelinated axons at P180 resulting in a 66% reduction with respect to C rats (0.36×10^6 axons). In the CC of C rats, the number of myelinated axons increased from 1.76×10^3 axons at P12 to 3.34×10^6 axons at P184. In H rats, there were only 0.84×10^6 axons at P184 resulting in a 76% reduction with respect to C rats. This reduction was more important in the posterior sector of the CC (95%) than in the rest (on average 63%). Therefore these results show that thyroid hormones play an important role in the processes involved in the maturation of commissural axons.

Key words: Anterior commissure; Corpus callosum; Cerebral cortex; Myelin; Quantitative electron-microscopy

1. Introduction

It has been postulated that high mental functions are mostly localized in the cerebral cortex and that transfer of information between the cerebral hemispheres plays a crucial role in cognitive and associative processes, e.g. [12]. Hypothyroidism in developing rats (H rats) damages the cortical cytoarchitecture [8,14,16,17] and the maturation of individual neurons by affecting development of dendrites [14,18], synthesis of microtubule-associated proteins [11,34], distribution of dendritic spines [39], number of microtubules and their spatial arrangement [4]. In addition, changes in the pattern of callosal connections between visual [21], somatosensory [21] and auditory cortical areas [8] have been reported recently. All these changes must be relevant to the retardation of the acquisition of innate behavioural responses and delayed adaptative behaviour observed in H rats [15] and might be applicable to the mental retardation characteristic of early hypothyroidism in humans.

How thyroid hormones (TH), 3,5,3′-triiodothyronine (T3) and thyroxine or 3,5,3′,5′-tetraiodothyronine (T4), influence the normal development of neurons and glial cells is still under study. T3, the active form of TH, enters the nucleus and regulates gene expression by binding specific c-erbA receptors [10,31]. The expression of these receptor mRNAs in the rat brain is very specific in the developing rat brain [10,31]. In H rats, the expression of myelin-associated glycoprotein (MAG), proteolipid protein (PLP) and myelin basic protein (MBP) in oligodendrocytes is significantly altered [19,33]. Other metabolic alterations have also been observed in H rats, resulting in changes in the maturation of neurons and glial cells during development (see Dussault and Ruel [13] for review).

In the development of corticocortical interhemispheric connections axons that will form permanent connections must find their target cells and then be stabilized and

[1] Some of these data have been first presented at the 14th Annual Meeting of the European Neuroscience Association, held in Cambridge, UK, on 8–12 September, 1991.

* Corresponding author. Fax: (34) (6) 565 8511.

SSDI 0166-4328(94)00035-E

mature. It has been postulated that stabilization and maturation of axons are interrelated [27]. This hypothesis can be tested in H rats in which many aspects of axonal maturation are severely affected. However, there are few data on the number of axons in the forebrain commissures of C and H rats [22,24]. In this report, we have quantified the number of axons in the CA and the CC in C and H rats. These results have been compared with those concerning the organization of callosal connections [8,21].

2. Materials and methods

2.1. Experimental hypothyroidism

Pregnant Wistar rats receibed an antithyroid treatment with 0.02% methimazole (Sigma; MMI) in the drinking water. Treatment began at embryonic day 14 (E14; vaginal plug appeared at E0) and continued after birth (= E22), until postnatal day 9 (P9). From P9 onwards (3 days after thyroidectomy, see below), 1% calcium gluconate was added to the MMI solution and it was maintained until the rats death. In addition to the MMI treatment, ether anaesthetized rats were subsequently thyroidectomized at P6 following a procedure already described [44]. Litters were equated to 8 pups at P8.

Hypothyroidism derived from thyroidectomy was assessed by control of body weight (b.wt.) and from tissue determination of TH by specific radioimmunoassays adapted for rat samples [35]. In H rats, b.wt. increased slightly with age from 20 g at P10 to 150 g at P180–184 and remained well below C values (463 g at P180; Fig. 1). At all ages, the concentrations of TH were lower in H than in C rats (Fig. 2). In H rats, there was on average 0.48 ± 0.28 ng of T3/g of tissue (1.35 ± 0.15 ng/g in C rats) and 0.12 ± 0.08 ng of T4/g of tissue (1.5 ± 0.5 ng/g in C rats).

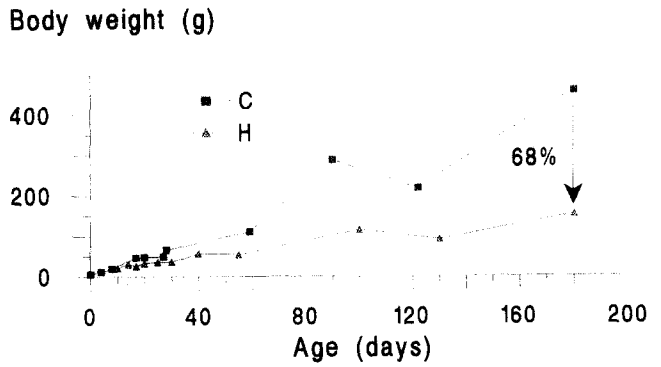

Fig. 1. Evolution of body weight (b.wt.) in C and H rats as a function of age. In C rats, there is an increase in b.wt. with age. In H rats, b.wt. increases slightly and remains well under C values.

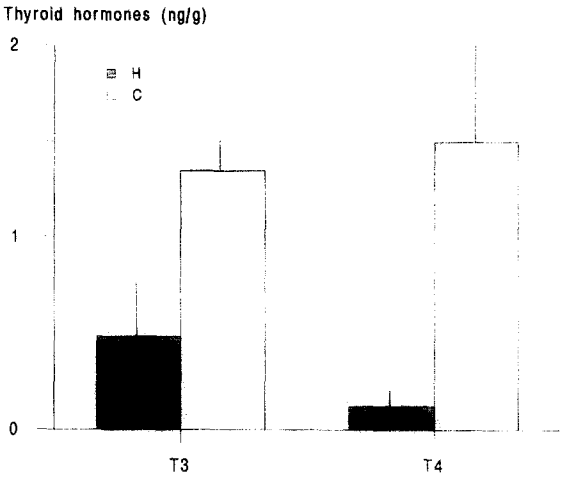

Fig. 2. Concentrations of T3 and T4 in C and H rats. Levels of T3 and T4 are lower in H than in C rats.

2.2. Conventional electron microscopy

In this study, 26 C and 24 H female rats were studied at ages between E18 and P184. C and H rats were deeply anaesthetized with Imalgène 100 (Rhône Mérieux; 0.15 ml/100 g b.wt.) and perfused with 0.002% $CaCl_2$ and 0.1M sucrose in 0.12M phosphate buffer (pH 7.3), containing paraformaldehyde and glutaraldehyde at variable concentrations depending on the age of the rat (for concentrations see Berbel and Innocenti [5]). The brain was removed and sagittally sectioned with a vibratome in 500 μm-thick slices. The most medial slice of each rat was postfixed in 2% osmium tetroxide and 7% glucose in 0.12 M phosphate buffer (pH 7.3) for 2 h, en bloc stained with 1% uranyl acetate in 0.08 M maleate buffer (pH 4.5–4.6) for 1.5 h, gradually dehydrated through ascending ethanols and embedded in Epon-Araldite. Ultrathin sections were stained with alcoholic uranyl acetate and aqueous lead citrate, and studied and photographed with a Zeiss EM10C/CR electron microscope.

2.3. Quantitative analysis

In the CC, axons were counted following a procedure already described [5]. Each CC was divided into three sectors (anterior, middle and posterior). The transversal area of each sector was measured in outlines made from semithin-sections obtained near the mid-sagittal plane. Differently from this previous study, each sector was randomly microphotographed at 16,000×. In total, 20 microphotographs (free of blood wessels and cell bodies) of 29 μm^2 mean area each were taken per sector. From these microphotographs, the mean density of processes was calculated. The estimation of the total number of processes per sector was obtained multiplying the mean density of

processes per sector by the area of each sector (free of blood wessels and cell bodies). The total number of processes of the CC was estimated by adding the number of processes of each sector. For the estimation of the total number of axons in the AC we used a similar procedure. The AC was divided into 20 consecutive square sectors. Approximately in the centre of each sector a microphotograph (free of blood wessels and cell bodies) at 16,000 × was taken covering a mean area of 29 μm^2. The total number of axons was estimated multiplying the mean density of axons by the mid-transversal area (free of blood wessels and cell bodies) of the AC obtained from semithin sections. Due to the low density of myelinated axons found between P12 and P55 (see Results), for the calculation of the density of myelinated axons, the AC was sampled in 10 evenly spaced microphotographs taken at 2,500 × and covering an area of 267 μm^2 each one.

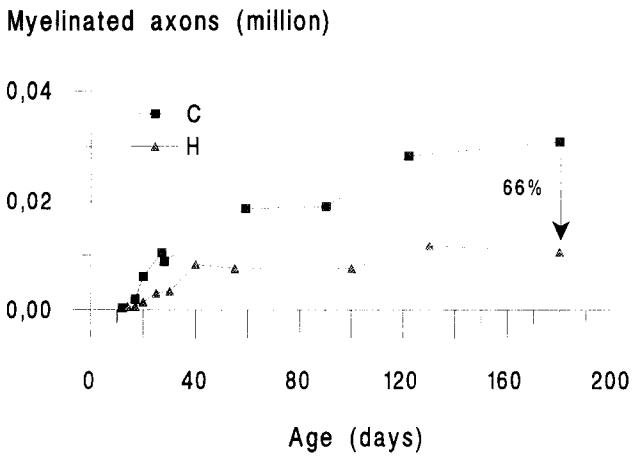

Fig. 4. Estimated number of myelinated axons in the AC as a function of age in C and H rats. Note the rapid increase in axon number between P12 and P59 in C rats. In H rats, a mean 66% reduction in the number of myelinated axons is observed at P180.

3. Results

3.1. Anterior commissure

In the AC, the total number of axons (unmyelinated and myelinated) was similar in C and H rats (Fig. 3). In C rats, the number of axons rapidly increased from E18 (168,500 axons) to P12 (1,155,700 axons) and remained almost constant after (964,400 axons at P180). A similar number of axons was observed in H rats, there were 931,500 axons at P14 and 841,800 axons at P180 (Fig. 3).

The number of myelinated axons was strongly reduced in the AC of H rats (Fig. 4). Myelinated axons were firstly observed at P12 in C and at P14 in H rats. In C rats, the number of myelinated axons rapidly increased from P12 (3,300 axons; 0.3%) to P59 (186,900 axons; 17.4%) and more slowly after (309,400 axons at P180; 32%). In H rats, the number of myelinated axons increased from P14 (6,000 axons; 0.64%) to P40 (83,700 axons; 7.6%) and slightly after (106,900 axons at P180; 12.7%), resulting in a 66% reduction at P180 with respect to C rats (Fig. 4).

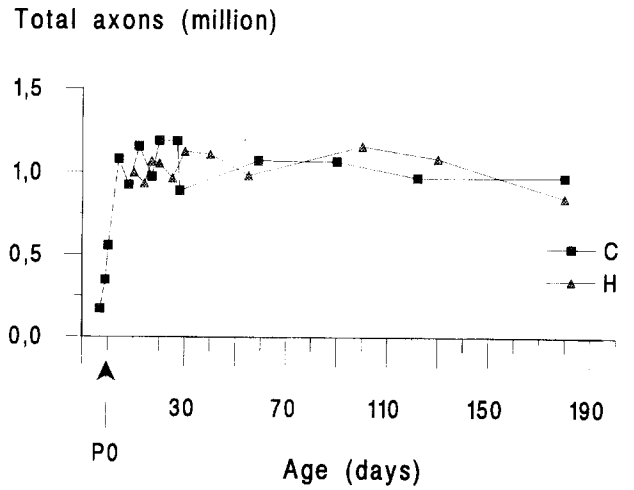

Fig. 3. Estimated number of axons in the AC as a function of age in C and H rats. The axon number is similar between C and H rats. It rapidly increases between E18 and P12 and then remains almost constant.

3.2. Corpus callosum

In all sectors of the CC, the number of myelinated axons was strongly reduced in H rats (Fig. 5). In C rats, myelinated axons were firstly observed in the anterior and medial sectors at P12 and in the posterior sector at P17. In H rats, they were observed in all sectors at P14. In C rats, the number of myelinated axons rapidly increased in the anterior and medial sectors from P12 (1,765 axons; 0,01%) to P59 (889,013 axons; 6%) and more slowly after (1,017,006 axons at P180; 8.6%; Fig. 5a,b). In the posterior sector, they progressively increased in number from P17 (47,324 axons; 0.3%) to P184 (1,355,980 axons; 11.3%; Fig. 5c). In H rats, the number of myelinated axons progressively increased in the anterior and medial sectors from P17 (9,280 axons; 0.06%) to P184 (381,773 axons; 2.8%), resulting in a 63% mean reduction at P184 with respect to C rats (Fig. 5a,b). In the posterior sector, they only increased in number from P17 (2,434 axons; 0.03%) to P184 (78,235 axons; 0.5%), resulting in a 95% reduction at P184 with respect to C rats (Fig. 5c). The proportion of myelinated axons was lower in the posterior sector than in the rest of the CC. At P184, the overall mean reduction in the number of myelinated axons was 76% (Fig. 5d).

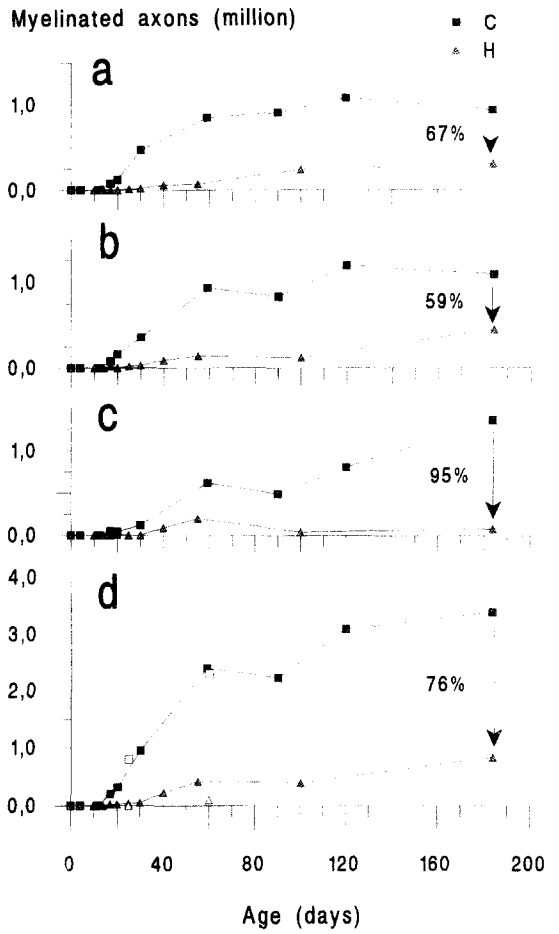

Fig. 5. Estimated number of myelinated axons in the anterior (a), middle (b) and posterior (c) sectors of the CC as a function of age in C and H rats. Totals are shown in "d". Note the rapid increase in axon number between P4 and P59 in C rats in all sectors of the CC. In H rats, a mean 76% reduction in the number of myelinated axons is observed at P184 (d); this reduction is still more important in the posterior sector of the CC. Open symbols in "d" represent data from Gravel et al. [22].

4. Discussion

4.1. Experimental hypothyroidism

As soon as E16, nuclear TH receptors in the brain reaches normal foetal level, approx. 30% of the adult [36]. In this study, MMI was administered to H rats from E14 onwards, before the onset of neocortical neurogenesis at E16 [9]. In H rats, the levels of TH in brain tissue were low. However, some amounts of T3 were still present (Fig. 2) which may have an exogenous origin from food-pellets, obtained from animal proteins, as previously discussed [8].

In addition to TH, other hormones and hormone-dependent metabolites are unbalanced in H rats [1,2,15, 32]. In particular, there is a decrease in growth hormone which may produce some brain damage and is the main cause of arrested body growth [25,26,40]. Furthermore, H rats may be undernourished which in turn may also induce mental retardation [3].

4.2. Evolution of number of commissural axons

The number of axons progressively increased in the AC with the age in C and H rats, and this also occoured in the anterior limb of the AC of the mouse [41]. Experiments using tracers [30] describe also a progressive development of the AC in the hamster. In contrast, in the CC of the cat [5], monkey [29] and rat [22], and in the AC of the monkey [28] there was an overproduction of axons followed by an important loss of transitory axons. However, in the AC of the rat it might also occur a significant loss of transitory axons during development, compensated by an increase of commissural axons at the same time. Such increment of new axons should, however, be tested in further experiments.

4.3. Thyroid hormones and axonal maturation

In C rats, our data on the number of myelinated axons agree with those obtained by Gravel et al. [22] at P25 and P60. However, in H rats, Gravel et al.'s estimates are well below ours (e.g. 88,000 axons at P60 [22] vs. 421.000 axons at P55, this study). Our estimates might be influenced by the TH content in H rats, but they are probably more affected by the sampling procedure. In the present study, due to the low density of myelinated axons observed in C and H rats between P12 and P55, the sampling area was increased to 267 μm^2 and the number of samples per rat increased to 10. Therefore, probably our data are more precise estimates of the total number of myelinated axons although some differences between rats must not be excluded.

In a parallel study [8] using horseradish peroxidase alone (HRP) or coupled to wheat germ agglutinin (WGA-HRP), it has been observed that auditory callosally projecting neurons were mostly found in deeper cortical layers and that they were more densely and evenly distributed in H than in C rats. In addition, axons anterogradely labelled from the contralateral hemisphere acquired an abnormal distribution between layers I and III in H rats [8]. Results in some respect similar to these were observed also in callosal projections of the visual and somatosensory cortical areas [21].

In H rats, the density of callosally projecting neurons was significantly increased [8]. This increase in the density of retrograde labelled callosal neurons in the auditory cortex might simply reflect an overall increase in cortical cell density in H rats, as has been observed in other cortical areas [17,18]. Actual estimates of cell numbers in the auditory areas are difficult to obtain since their borders are not clearly visible in Nissl-stained sections.

In rats, as in many other mammals, the adult distribution of callosally projecting neurons is due to a localized

and selective loss of callosal projections (for review see Innocenti [27]). Therefore, the even distribution of callosally projecting neurons observed in adult H rats might be the result of the maintenance of transitory juvenile callosal projections [8,21]. The development of permanent callosal axons implies the transition from a juvenile-labile to an adult-stable state which for most of the axons involve a series of consecutive developmental steps including the maturation of several cytoskeletal proteins [20,23,37,38], the increase in axon calibre [6] and the myelination of some of them [5]. In H rats, the maturation of some cytoskeletal components is delayed or does not occur [11,21,34] and the same applies for the growth of axon calibre [24] and myelination [22,24]. Therefore, in H rats, stabilization of juvenile axons may be dissociated from cytoskeletal maturation and myelination which were previously thought to be necessarily linked [27], see [8] for discussion.

In the forebrain commissures, maturation of oligodendrocytes might also be affected in H rats as occurs in the cerebral cortex. In the cortex, the expression of MAG, PLP and MBP in oligodendrocytes is strongly reduced [19,33] (see Introduction). In addition, in H rats [7], the total number of oligodendrocytes is reduced in the AC and probably the same occurs in the CC. These data indicates that the decrease in the number of myelinated axons observed in H rats is not exclusively due to an impairment of axonal maturation but also to a damage in glial maturation and proliferation.

These changes can affect the structure and function of sensory areas connected through the cerebral commissures. At least in part, they might be responsible for deafness [42] and audiogenic seizures [43] associated to hypothyroidism.

Acknowledgements

We are indebted to Dr. G.M. Innocenti for critical comments on the manuscript. We thank E. Gutierrez, M.D. Segura and K. Hernández for technical assistance. A. Guadaño-Ferraz was recipient of a pre-doctoral fellowship of the "Generalitat Valenciana". This work was supported by a grant of the Spanish DGICYT PB90-0561 to P. Berbel.

References

[1] Balázs, R., Influence of metabolic factors on brain development, *Brit. Med. Bull.*, 30 (1974) 126–134.

[2] Balázs, R., Lewis, P.D. and Patel, A.J., Effects of metabolic factors on brain development. In M.A.B. Brazier (Ed.), *Growth and Development of the Brain*, Raven, New York, 1975, pp. 83–115.

[3] Balázs, R., Lewis, P.D. and Patel, A.J., Nutritional deficiencies and brain development. In F. Falkner and J.M. Tanner (Eds.), *Human Growth, Vol. 3*, Plenum, New York, London, 1979, pp. 415–480.

[4] Berbel, P., Escobar del Rey, F., Morreale de Escobar, G. and Ruiz-Marcos, A., Effect of hypothyroidism on the development of cortical dendritic spines. An electron microscopic study, *Ann. Endocrinol.*, Suppl. 44 (1983) 16A.

[5] Berbel, P. and Innocenti, G.M., The development of the corpus callosum in cats: a light- and electron-microscopic study, *J. Comp. Neurol.*, 276 (1988) 132–156.

[6] Berbel, P., Innocenti, G.M., Prieto, J.J. and Kraftsik, R., A quantitative study on the development of the cytoskeleton of callosal neurons in cats, *Eur. J. Neurosci.*, Suppl. 2 (1989) 47.

[7] Berbel, P., Guadaño-Ferraz, A., Angulo, A. and Rueda, J., The development of glial cells in the anterior commissure in normal and hypothyroid rats, *Eur. J. Neurosci.*, Suppl. 4 (1991) 102.

[8] Berbel, P., Guadaño-Ferraz, A., Martínez, M., Quiles, J.A., Balboa, R. and Innocenti, G.M., Organization of auditory callosal connections in hypothyroid rats, *Eur. J. Neurosci.*, 5 (1993) 1465–1478.

[9] Berry, M., Rogers, A.W. and Eayrs, J.T., The pattern and mechanism of migration of the neuroblasts of the developing cerebral cortex, *J. Anat.*, 98 (1964) 291–292.

[10] Bradley, D.J., Towle, H.C. and Young III, W.S., Spatial and temporal expression of α- and β-thyroid hormone receptor mRNAs, including the β2-subtype, in the developing mammalian nervous system, *J. Neurosci.*, 12 (1992) 2288–2302.

[11] Cerezo, J.R., Guadaño-Ferraz, A., Riederer, B.M. and Berbel, P., Development of cytoskeletal components in commissural axons of normal and hypothyroid rats, *Eur. J. Neurosci.*, Suppl. 4 (1991) 270.

[12] Doty, R.W., Ringo, J.L. and Lewine, J.D., Forebrain commissures and visual memory: a new approach, *Behav. Brain Res.*, 29 (1988) 267–280.

[13] Dussault, J.H. and Ruel, J., Thyroid hormones and brain development, *Annu. Rev. Physiol.*, 49 (1987) 321–334.

[14] Eayrs, J.T., The cerebral cortex of normal and hypothyroid rats, *Acta Anat.*, 25 (1955) 160–183.

[15] Eayrs, J.T., The status of the thyroid gland in relation to the development of the nervous system, *Animal Behav.*, 7 (1959) 1–17.

[16] Eayrs, J.T. and Taylor, S.H., The effect of thyroid deficiency induced by methyl thiouracil on the maturation of the central nervous system, *J. Anat.*, 85 (1951) 350–358.

[17] Eayrs, J.T. and Horn, G., The development of cerebral cortex in hypothyroid and starved rats, *Anat. Rec.*, 121 (1955) 53–61.

[18] Eayrs, J.T. and Goodhead, B., Postnatal development of the cerebral cortex in the rat, *J. Anat.*, 93 (1959) 385–402.

[19] Farsetti, A., Mitsuhashi, T., Desvergne, B., Robbins, J. and Nikodem, V.M., Molecular basis of thyroid hormone regulation of myelin basic protein gene expression in rodent brain, *J. Biol. Chem.*, 266 (1991) 23226–23232.

[20] Figlewicz, D.A., Gremo, F. and Innocenti, G.M., Differential expression of neurofilament subunits in the developing corpus callosum, *Dev. Brain Res.*, 42 (1988) 181–189.

[21] Gravel, C. and Hawkes, R., Maturation of the corpus callosum of the rat: I. Influence of thyroid hormones on the topography of callosal projections, *J. Comp. Neurol.*, 291 (1990) 128–246.

[22] Gravel, C., Sasseville, R. and Hawkes, R., Maturation of the corpus callosum of the rat: II. Influence of thyroid hormones on the number and maturation of axons, *J. Comp. Neurol.*, 291 (1990) 147–161.

[23] Guadaño-Ferraz, A., Riederer, B.M. and Innocenti, G.M., Developmental changes in the heavy subunit of neurofilaments in the corpus callosum of the cat, *Dev. Brain Res.*, 56 (1990) 244–256.

[24] Guadaño-Ferraz, A., Berbel, P., Balboa, R. and Innocenti, G.M., The development of the anterior commissure in normal and hypothyroid rats, *Eur. J. Neurosci.*, Suppl. 4 (1991) 225.

[25] Hamburg, M., The role of thyroid and growth hormone in neuro-

genesis. In A.A. Moscona and A. Monroy (Eds.), *Current Topics in Development Biology, Vol. 4*, Academic Press, London, 1969, pp. 109–148.

[26] Hervás, F., Morreale de Escobar, G. and Escobar del Rey, F., Rapid effects of single small doses of L-thyroxine and triiodo-L-thyronine on growth hormone, as studied in the rat radioimmunoassay, *Endocrinology*, 97 (1975) 91–101.

[27] Innocenti, G.M., The development of projections from cerebral cortex, *Prog. Sens. Physiol.*, 12 (1991) 65–114.

[28] LaMantia, A.S. and Rakic, P., The number, size, myelination, and regional variation of axons in the corpus callosum and anterior commissure of the developing Rhesus monkey, *Proc. Soc. Neurosci.*, 10 (1984) 1081.

[29] LaMantia, A.S. and Rakic, P., Axon overproduction and elimination in the corpus callosum of the developing Rhesus monkey, *J. Neurosci.*, 10 (1990) 2156–2175.

[30] Lent, R. and Guimarães, R.Z.P., Development of paleocortical projections through the anterior commissure of hamsters adopts progressive, not regressive, strategies, *J. Neurobiol.*, 22 (1991) 475–498.

[31] Mellström, B., Naranjo, J.R., Santos, A., González, A.M. and Bernal, J., Independent expression of the α and β c-erb*A* genes in developing rat brain, *Mol. Endocrinol.*, 5 (1991) 1339–1350.

[32] Morreale de Escobar, G., Escobar del Rey, F. and Ruiz-Marcos, A., Thyroid hormone and the developing brain. In J.H. Dussault and P. Walker (Eds.), *Congenital Hypothyroidism*, Marcel Dekker, New York, 1983, pp. 85–126.

[33] Muñoz, A., Rodríguez-Peña, A., Pérez-Castillo, A., Ferreiro, B., Sutcliffe, J.G. and Bernal, J., Effects of neonatal hypothyroidism on rat brain gene expression, *Mol. Endocrinol.*, 5 (1991) 273–280.

[34] Nunez, J., Couchie, D. Aniello, F. and Bridoux A.M., Regulation by thyroid hormone of microtubule assembly and neural differentiation, *Neurochem. Res.*, 16 (1991) 975–982.

[35] Obregón, M.J., Morreale de Escobar, G. and Escobar del Rey, F., Concentrations of triiodo-L-thyronine in the plasma and tissues of normal rats as determined by radioimmunoassay: comparison with results obtained by an isotopic equilibrium technique, *Endocrinology*, 103 (1978) 2145–2153.

[36] Pérez-Castillo, A., Bernal, J., Ferreiro, B. and Pans, T., The early ontogenesis of thyroid hormone receptor in the rat fetus, *Endocrinology*, 117 (1985) 2457–2461.

[37] Riederer, B.M., Guadaño-Ferraz, A. and Innocenti, G.M., Difference in distribution of microtubule-associated proteins 5a and 5b during the development of cerebral cortex and corpus callosum in cats: dependence on phosphorylation, *Dev. Brain Res.*, 56 (1990) 235–243.

[38] Riederer, B.M. and Innocenti, G.M., Differential distribution of tau proteins in developing cat cerebral cortex and corpus callosum, *Eur. J. Neurosci.*, 3 (1991) 1134–1145.

[39] Ruiz-Marcos, A., Quantitative studies of the effects of hypothyroidism on the development of the cerebral cortex. In G.R. DeLong, J. Robbins and P.G. Condliffe (Eds.), *Iodine and the Brain*, Plenum, New York, 1989, pp. 91–102.

[40] Seo, H., Martino, E., Refetoff, S. and Vassart, G., The differential stimulatory effect of thyroid hormone and estrogen on growth hormone and prolactin is due to induction of specific mRNAs, *Endocrinology*, 103 (1978) 1506–1509.

[41] Sturrock, R.R., A quantitative electron microscopic study of myelination in the anterior limb of the anterior commissure of the mouse brain, *J. Anat.*, 119 (1975) 67–75.

[42] Trotter, W.R., The association of deafness with thyroid disfunction, *Brit. Med. Bull.*, 16 (1960) 92–98.

[43] Van Middlesworth, L. and Norris, C.H., Audiogenic seizures and cochlear damage in rats after perinatal antithyroid treatment, *Endocrinology*, 106 (1980) 1686–1690.

[44] Zarrow, M., Yochim, J.M. and McCarthy, J.L., *Experimental Endocrinology: A Sourcebook of Basic Techniques*, Academic Press, London, New York, 1964, pp. 241–242.